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Interaction of Carbohydrate Binding Sites on Concanavalin A-Agarose with Receptors on Adipocytes Studied by Buoyant Density Method†

Howard M. Katzen* and Denis D. Soderman

ABSTRACT: The interaction of concanavalin A (Con A) with isolated adipocytes was studied using Con A-Sepharose beads in the affinity binding buoyant density method previously used to study insulin receptors. Free Con A-Sepharose beads could be separated from the bound beads (cell–bead complexes) by sedimentation of the high density beads and floatation of the low density complexes. Sedimented and total beads could be determined by counting the radioactivity associated with [^{125}I]Con A coupled in tracer amounts to the beads. Various lines of evidence demonstrated the high specificity of binding. Soluble Con A, but neither insulin nor any of the other proteins tested, inhibited and reversed the binding of Con A-Sepharose to the cells. Whereas treatment of Con A- (and insulin-) derivatized beads with anti-insulin antiserum, and cells with trypsin, readily inhibited binding of insulin-Sepharose to cells, neither treatment inhibited Con A-Sepharose binding. According to the relative extents of inhibition and reversal of binding exhibited by 15 different carbohydrates, the saccharide binding sites on Con A-Sepharose appeared virtually identical with the known sites on free Con A. Protein-containing components of cell ghosts that were solubilized with Triton X-100 appeared to correspond to the Con A-Sepharose receptor sites on the basis of their ability to bind to Con A-Sepharose columns, be eluted with methyl α-D-mannopyranoside (MeMan) and be precipitated by the free lectin and redissolved by MeMan. According to (a) Normarski interference contrast microscopic examination of the topographical distribution of Con A-Sepharose beads and cells surrounding and bound to each other, and (b) absence of any apparent morphological changes in the cells due to binding, it is suggested that extensive clustering ("cap" or "macropatch" formation) of Con A receptors did not occur on the adipocyte as a consequence of the interaction of the cells with the Con A-Sepharose beads.

Although plant lectins have been widely used to probe the structure and function of cell surfaces of a variety of mammalian cells (reviewed by Lis and Sharon, 1973), there have been relatively few reports on the adipocyte as a target cell for these studies. Interest in the effects of, and binding sites for, lectins on the adipocyte has recently been stimulated by the finding of insulin-like activity exhibited by the interaction of concanavalin A[1] with these cells (Czech and Lynn, 1973; Cuatrecasas and Tell, 1973).

Persistent doubts expressed (Lambert et al., 1972; Hamlin and Arquilla, 1974) about the validity of ^{125}I-radioiodinated insulin as a biologically active and relevant ligand to study the insulin receptor prompted us to search for alternative ligands and to develop new methodology to study membrane receptor sites. Our previous findings of specific and reversible binding of insulin-agarose (insulin-Sepharose) beads to intact isolated adipocytes that led to the development of an "affinity-binding buoyant density" assay procedure to study insulin receptor sites (Katzen and Soderman, 1973; Soderman et al., 1973), suggested that an analogous procedure, utilizing Con A-Sepharose, could be applied to study the interaction of Con A with these cells. The particular advantage of adipocytes as a target for Con A-Sepharose beads is the ability of these low density cells in physiological media to float the derivatized beads bound to the cells. The unbound beads would otherwise sediment.

In earlier studies with immobilized Con A, it was found that intact cells specifically bound to Con A-derivatized nylon fibers or agarose beads could not be released (eluted) without distortion of the cells by physical or mechanical methods (Edelman et al., 1971). Because of these and other known limitations of affinity chromatographic-like binding (Cuatrecasas and Anfinsen, 1971; Shaltiel and Er-el, 1973; Katzen and Vlahakes, 1973), studies on any specific type of cells require that a determination be made of the specificity and reversibility of the binding of the derivatized support to those cells. The present study is intended to demonstrate the specific and reversible binding of Con A-Sepharose beads to intact adipocytes for the purpose of applying the affinity-binding buoyant density procedure to the study of the interaction between the saccharide binding sites on Con A and

† From the Department of Biochemical Endocrinology, Merck Institute for Therapeutic Research, Rahway, New Jersey 07065. *Received December 20, 1974.*

[1] Abbreviations used are: Con A, concanavalin A; Con A (or insulin)-Sepharose, Con A (or insulin) covalently coupled to Sepharose 4B (agarose); MeMan, methyl α-D-mannopyranoside; AIS, anti-insulin antiserum.

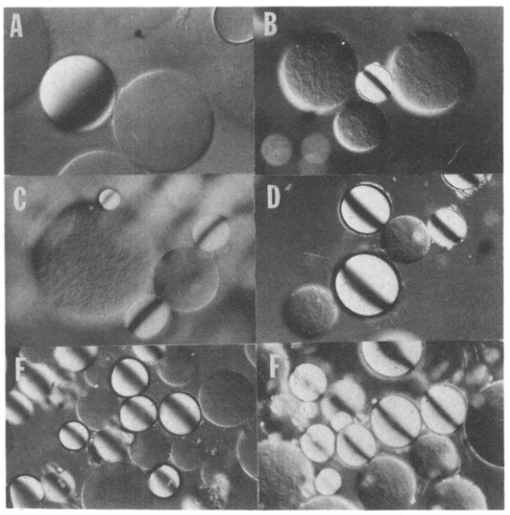

FIGURE 1: Nomarski interference contrast microscopy (×100) of Con A-Sepharose bead–cell complexes comprising two (A) and three (B) beads bound to a cell, two (C) and three (D) cells bound to a bead, and more concentrated mixtures of cells and beads bound to each other (E and F). Adipocytes each appear with a distinct dark shadowy band across a clear white and smooth surface, while beads, which are usually larger than cells, appear with dark, rough, and porous surfaces devoid of the shadowy band.

the lectin receptor sites on the cells. The binding of the cells to the beads was also examined by Nomarski optics.

Materials and Methods

The fat cells (adipocytes) were derived from the distal half of the epididymal adipose tissue taken from male CD albino rats (Charles River). The rats weighed between 140 and 180 g and were allowed free access to Purina laboratory chow. Isolated adipocytes and their ghosts were prepared by the procedures of Rodbell (1964, 1967).

Con A-Sepharose (8 mg of Con A coupled/ml of packed beads) and soluble Con A were obtained from Pharmacia Fine Chemicals. Immediately prior to its use, Con A-Sepharose was washed thoroughly with 400 volumes of water, suspended in a large volume of 6 *M* guanidine-HCl for 0.5 hr, filtered, rewashed with water, and equilibrated with Krebs-Ringer phosphate buffer. When used in tracer amounts as a radioactive label coupled to Sepharose in the buoyant density procedure (Soderman et al., 1973), Con A was iodinated according to the modification by Freychet et al. (1971) of the procedure of Greenwood et al. (1963). Modified and unmodified saccharides were obtained from commercial sources where available, or were kindly provided by Dr. P. Durette of our Institute. Trypsin (two times crystallized) was a product of ICN Nutritional Biochemicals and soybean trypsin inhibitor was obtained from Worthington Biochemical Corp.

The affinity binding buoyant density procedure was conducted according to the method previously described (Soderman et al., 1973), except that Con A-Sepharose beads were used instead of insulin-Sepharose beads. Plastic tubes (5 ml, 12 × 75 mm) were used for all assays. For assays of inhibition of binding, varying amounts (in 0.1–0.2 ml) of inhibitors, in Krebs-Ringer PO_4 buffer, were added to 0.33 ml of packed beads immediately followed by the addition of freshly prepared packed cells at the designated ratio of cells to beads in a final volume of 1 ml with buffer. Immediately thereafter, the final mixture was gently but completely mixed for 5 sec and allowed to separate in a vertical position into three distinct layers without disturbance for 15 min at room temperature. The two top layers (floating cells bound or unbound to beads, and most of the clear intermediate layer) were aspirated off and discarded, and the radioactivity associated with the sedimented beads was counted. For assays of dissociation of binding (reversal of binding in contradistinction to inhibition), the same amounts of cells and beads, except in a final volume of 0.9 ml, were mixed together and allowed to separate for 15 min under the conditions described above. Afterwards, 0.1 ml of the inhibitor was carefully added and the resultant mixture was gently remixed for a few seconds and again allowed to reseparate into distinct layers for 15 min. The sediment was then counted for radioactivity as described above.

Other materials and methods are as previously described (Katzen and Soderman, 1973; Soderman et al., 1973).

Results

Interference Contrast Microscopy of Cell–Bead Interactions and Morphology. Intact, viable adipocytes were found to bind readily to Con A-Sepharose beads in isotonic media (Figure 1) in a manner analogous to that seen for insulin-Sepharose under identical conditions (Katzen and Soderman, 1973; Soderman et al., 1973). That cells and Con A-derivatized beads were, indeed, firmly bound to each other could be demonstrated by disturbing the slide under the microscope and observing the movements of the cell–bead complexes. Clearly each of the complexes seen in Figure 1 drifted as a unit. As previously demonstrated for the binding of insulin-Sepharose to adipocytes, the cell–bead complexes would either float or sediment, depending upon their buoyant densities. The net buoyant densities were a function of the number of cells bound per bead which, in turn, depended upon the ratio of cells to beads present in the mixture.

The examples in Figure 1 are also intended to demonstrate that individual cells were each capable of binding more than one Con A-Sepharose bead at a time, and that this multiplicity of binding appeared identical with the ability of each bead to bind simultaneously many cells. For this reason, Figure 1 only represents examples of cell–bead complexes obtained after diluting and shaking the mixtures to obtain only a relatively few beads and cells bound to each other. In the initially concentrated mixtures, cells were always seen to be completely surrounded by beads to which the cells were bound. On the basis of numerous observations by Nomarski optics, there appeared to be an even topographical distribution of receptor sites on the surface of the cells, similar to the distribution of binding sites around the Con A-Sepharose beads. There thus appeared to be no restrictions, other than availability of space, on the number of beads a cell could bind.

In addition to this apparent absence of Con A-Sepharose-induced segregation of receptor sites, there also appeared to be no morphological changes in the cells resulting from their interaction with these beads. Clearly, the adipocytes retained their spherical shape.

Assay of Cell-Derivatized Bead Binding. By counting the [^{125}I]Con A coupled to the beads as a tracer label, the sedimented beads with no (or few) cells bound to them could be measured and compared with the total beads present after mixing various proportions of cells to beads (Table I). Analogous to the situation with the binding of insulin-Sepharose to adipocytes (Soderman et al., 1973), decreas-

Table I: Binding Capacities of Trypsin-Treated Adipocytes and Anti-insulin Antiserum-Treated Derivatized Agarose Beads.

Treatment[a]	Cell–Bead Ratio[d]	Sedimented Beads[e]	
		Con A-Sepharose	Insulin-Sepharose
		(cpm and percent of total cpm[f])	
None	0.6	635 (1.2%)	2223 (13%)
5 μl AIS[b]	0.6	618 (1.1%)	13849 (86%)
100 μl AIS	0.6	652 (1.2%)	15952 (99%)
None	0.4	6354 (12%)	4128 (26%)
5 μl AIS[b]	0.4	6325 (12%)	15792 (98%)
100 μl AIS	0.4	7516 (14%)	15987 (99%)
None	0.8	431 (5.0%)	507 (14%)
1.0 μg trypsin[c]	0.8	452 (5.2%)	797 (21%)
2.5 μg trypsin	0.8	464 (5.4%)	1136 (30%)
5.0 μg trypsin	0.8	433 (5.0%)	2158 (58%)
1.0 mg trypsin	0.8	425 (4.9%)	3424 (92%)
4.0 mg trypsin	0.8	525 (6.1%)	3586 (96%)

[a] "None" refers to treatment of beads or cells in the absence of the AIS or trypsin (see below). [b] Guinea pig antiserum to bovine insulin (AIS) was mixed with derivatized beads in 0.3 ml of Krebs-Ringer PO_4 buffer (pH 7.4) and allowed to stand 5 min at room temperature. [c] Cells were incubated with the designated (per ml) amounts of trypsin in the buffer for 10 min at 37°, after which excess soybean trypsin inhibitor was added. [d] Adipocytes were added to the treated beads, or treated cells to beads, at the designated proportion of cells to 0.33 ml of settled beads to a final volume of 1.0 ml, after which the mixtures were rapidly but gently mixed and allowed to separate for 15 min. [e] The supernatant and most of the infranatant were discarded by aspiration and the sediment remaining was counted. [f] The "total cpm" represents radioactivity associated with the total amount of beads initially mixed with cells.

Table II: Relative Abilities of Proteins to Inhibit and Reverse Binding of Con A-Sepharose to Adipocytes.

Protein[a]	Percent of Total Beads Sedimented by Protein Added	
	Before Binding[c] (above base line)	After Binding[d] (normalized)
Insulin (5×10^{-4} M)[b]	0	0
BPA (4%)	0	0
Gelatin (4%)	0	0
Con A (40 μg/ml)	0	0
Con A (400 μg/ml)	5	3
Con A (1.0 mg/ml)	35	
Con A (4.0 mg/ml)	99	95

[a] Concentrations designated are in final volume of 1.0 ml in which the ratio of cells to beads was 0.6. BPA is bovine plasma albumin. [b] This concentration approached the maximum solubility of insulin. [c] This represents inhibition of binding; protein was incubated with beads *prior to* addition of cells as in Table I; the "base line" sedimentation occurring in the absence of added protein was subtracted from the sedimented radioactivity in the presence of added protein. [d] This represents reversal of binding; protein was added *after* the cell–bead complexes were formed and values were calculated from radioactivity measurements of the sedimented beads, as described in Figure 2.

ing the proportion of cells to Con A-Sepharose beads resulted in an increase in the average buoyant density of the resultant complexes and therefore an increase in the number of beads that sedimented. The degrees of sedimentation of beads that were determined from radioactivity measurements always corresponded well with those values estimated from visual inspection of the volume of the sedimented layer compared with the volume of the floating layer in each assay tube.

Protein Specificity of Binding. Anti-insulin antiserum (AIS) readily inhibits the binding of insulin-Sepharose beads to adipocytes (Soderman et al., 1973). To show that the binding capacity of Con A-Sepharose is not due to nonspecific protein–protein interactions unrelated to Con A's saccharide binding sites, the effect of AIS on Con A-Sepharose binding to adipocytes was compared to its effect on insulin-Sepharose at various ratios of cells to beads (Table I). While AIS even at the low concentration completely inhibited the binding capacity of insulin-Sepharose, none of the concentrations tested had any significant effect on the ability of Con A-Sepharose to bind the cells. Likewise, while trypsin treatment of adipocytes readily incapacitates insulin receptors (Kono and Barham, 1971; Soderman et al., 1973), even at concentrations as low as 1 μg/ml (Table I), the binding capacity of the Con A receptors remained undiminished up to the highest concentration of trypsin tested (4 mg/ml).

According to the principle of affinity chromatographic binding (Cuatrecasas and Anfinsen, 1971; Shaltiel and Erel, 1973), the ligand in the soluble state should competitively inhibit the binding of immobilized ligand. In Table II it can be seen that while neither insulin, bovine plasma albumin, nor gelatin had any visible effects on the ability of Con A-Sepharose beads to bind the cells, soluble Con A at lower concentrations exhibited significant inhibition.

Reversibility of Interaction and Specificity of Saccharide Binding Sites. To demonstrate the reversibility of the binding of adipocytes to the Con A-Sepharose beads, the specific ability of free Con A to release the cells from the beads was also tested. Under these conditions (Table II, "after binding"), free Con A was added to the mixture of the cells and beads *after* all of the beads were bound to, and floated with, the cells. There the soluble Con A specifically reversed the binding, whereas the other proteins tested at higher concentrations had no effect. This was best illustrated by the sedimentation of nearly all of the beads after the addition of the Con A at a final concentration of 4 mg/ml.

On the basis of the following considerations, these seemingly high concentrations of free Con A would be expected to be required to inhibit or reverse the binding. First, because the derivatized beads contain 8 mg of Con A coupled/ml of packed Sepharose (see Materials and Methods), the diluted reaction mixtures reported in Table II each actually contained the relatively high concentration of about 2.6 mg of immobilized lectin/ml. Secondly, the local concentration of immobilized Con A at the site of binding to the cell surface would be anticipated to be even higher. The relatively large number of receptor–ligand interactions simultaneously occurring at each cell–bead juncture would likely lend itself to the requirement for a correspondingly high concentration of soluble ligand to act on a sufficient number of the bonds to completely inhibit or dissociate each cell from each bead.

It should also be noted in Table II that, according to measurements of the volume of the floating layer remaining after sedimentation of all of the derivatized beads by soluble Con A, virtually all of the cells originally present in the mixture remained floating. This indicated that under these conditions, soluble Con A-induced sedimentation of beads does not accompany sedimentation of cells bound to beads which, in turn, demonstrates complete dissociation of beads from cells. Under identical conditions, much higher concen-

Table III: Relative Inhibitory Effects of Various Saccharides: Comparison of Results from Buoyant Density Method with Dextran Precipitin Method[a]

Saccharide	Relative Potency[b] of Inhibition of: Con A-Sepharose to Adipocytes[c] (Normalized and % Sediment)	Free Con A to Dextran[d] (Normalized)
p-Aminophenyl α-D-glucopyranoside	100 (91%)	100
Methyl α-D-mannopyranoside	95 (86%)	81
N-Acetyl-D-glucosamine	64 (58%)	"Inhib."
D-Mannose	58 (53%)	37
Methyl α-D-glucopyranoside	47 (43%)	25
Maltose	22 (20%)	11
p-Aminophenyl β-D-glucopyranoside	13 (12%)	3.7
p-Aminophenyl α-D-galactopyranoside	11 (10%)	
D-Glucose	8.8 (8%)	2.2
p-Nitrophenyl α-D-galactopyranoside	5.5 (5%)	
N-Acetyl-D-mannosamine	0 (0%)	"Non-Inh."
3-*O*-Methylglucose	0 (0%)	"Non-Inh."
Lactose	0 (0%)	"Non-Inh."
D-Galactose	0 (0%)	"Non-Inh."
N-Acetyl-D-galactosamine	0 (0%)	"Non-Inh."

[a] The dextran precipitin method refers to the method employed, and relative values from results reported, by Goldstein and coworkers (Goldstein et al., 1965, 1974; Iyer and Goldstein, 1973) who measured the binding of free Con A to dextran. [b] Inhibitory effects from both methods are normalized relative to the effect of the most potent saccharide, which is assigned a value of 100. [c] This column refers to the inhibitory values normalized and also expressed (in parentheses) as the percentage of the total beads that sedimented as a result of the inhibitor added (at a final concentration of 0.1 *M*) before the addition of cells (ratio of 0.4 of cells to beads) in the buoyant density assay as described in Table I. [d] This column refers to the normalized values that we calculated from the concentrations that were reported (Iyer and Goldstein, 1973; Goldstein et al., 1974) to be required for 50% inhibition of the precipitation of dextran by free Con A. For those compounds for which they presented no quantitative values, Goldstein et al. 1965 recorded the saccharide as either an "inhibitor" ("Inhib.") or "non-inhibitor" ("Non-Inh.").

trations of the three proteins other than Con A had no ability to reverse the binding. It was previously shown that incomplete inhibition or incomplete reversal of binding can lead to the sedimentation of beads to which a few cells remain bound (Soderman et al., 1973).

Goldstein and coworkers had earlier established the specificity of the saccharide binding sites on soluble Con A directed to the lectin binding sites on soluble polysaccharides (Goldstein et al., 1965, 1974; Iyer and Goldstein, 1973). They tested the ability of various mono- and disaccharides and modified sugars to inhibit the precipitation of dextran by Con A. This afforded us the excellent opportunity to compare the specificity of the saccharide binding sites on Con A-Sepharose directed to intact adipocytes with their results (Table III).

In Table III it can be seen that MeMan was an excellent inhibitor that was more effective than the corresponding glucoside in both systems. Although the poor solubilities of the *p*-nitrophenyl α-D-glycoside of glucose and mannose and *p*-aminophenyl α-D-mannoside prevented them from being tested at the same concentrations as the others in Table III, we found that they exhibited up to 50% inhibition of binding at lower concentrations (about 0.01 *M*) at which they were soluble. In agreement with the results reported by Iyer and Goldstein (1973), the mannoside derivatives in these series proved to be the most potent. It is also interesting that although mannose (in the monosaccharide series) and the mannosides appeared as the most potent sugar moieties in their respective series, *N*-acetyl-D-mannosamine was a noninhibitor while *N*-acetyl-D-glucosamine was very potent in both the buoyant density and dextran precipitin systems.

Poor or noninhibitors of the interaction of soluble Con A with polysaccharide proved, in all cases, to have little or no effect on the binding capacity of Con A-Sepharose to adipocytes. Significantly, the relative inhibitory effects on Con A-Sepharose of the α and β-anomers of *p*-aminophenyl D-glucoside paralleled their relative inhibitory effects on free Con A. Using a concentration at which the less soluble *p*-nitrophenyl glucosides were soluble (1 m*M*; not shown), we also found the α anomer (normalized to 100) to be more potent than the β anomer (resulting in a normalized value of 26). This is also analogous to that reported by Iyer and Goldstein (1973).

For the same reasons noted in regard to Table II, relatively high concentrations of saccharides (0.001–0.1 *M*), in comparison to those required in the soluble Con A–soluble dextran system, were required to inhibit the interaction of cells with immobilized Con A. This is also consistent with the comparable amounts of MeMan (about 10 mg/ml) that have been used to elute glycoproteins from Con A-Sepharose affinity chromatographic columns (Bessler and Goldstein, 1973).

To examine further the reversibility of the binding of Con A-Sepharose to these cells, MeMan was tested at various concentrations for its ability to release the cells that were prebound to the derivatized beads (Figure 2). When this inhibitor was added at a final concentration greater than about 10^{-4} *M* 15 min after the cell–bead complexes had formed, it readily dissociated the cells from the beads in a dose–response manner. At about 3 m*M* MeMan, nearly 100% of the beads sedimented, while the cells remained floating. Testing (at 0.1 *M*) of the other saccharides reported in Table III under these conditions revealed a specificity of reversal of binding (not shown) that was virtually identical with the relative inhibitory effect seen in that table.

In other repeated experiments designed to document the existence in the adipocyte surface membrane of glycoproteins capable of exhibiting free Con A and Con A-Sepharose receptor-like properties, membrane preparations (Rodbell, 1967) were dissolved in 0.5% Triton X-100 in a manner identical with our previously described procedure (Katzen and Soderman, 1973). After passing this extract through an affinity chromatographic column of Con A-Sepharose, about 9–10% of the total solubilized protein-containing material was found to bind in repeated experiments. MeMan (0.3 *M*) completely and consistently eluted the bound protein-containing fraction. In addition, soluble Con A at 500 μg/ml was capable of precipitating between 10 and 11% of the total protein from the original extracts and, analogous to its ability to elute the protein from the column, 0.1 *M* MeMan readily redissolved the precipitate.

Discussion

The present study demonstrates the ability of isolated, in-

tact adipocytes to bind to specific saccharide-binding sites on Con A-Sepharose beads in a tight but reversible manner. The specificity of the saccharide binding sites on the beads was found to correspond exceedingly well to the specificity previously established (Goldstein et al., 1965, 1974; Iyer and Goldstein, 1973) for the saccharide binding sites on soluble Con A directed to the lectin-binding sites on free dextran.

This specific and reversible binding of Con A-Sepharose beads to buoyant cells also demonstrates the applicability of a new affinity binding buoyant density method for the study of the lectin-binding receptor sites on adipocytes, as well as for the binding sites on the lectin itself. We had previously described the ability of insulin receptors on intact adipocytes and membrane preparations, as well as a soluble receptor preparation, to bind tightly to various beaded insulin-Sepharose preparations in a specific and reversible manner (Katzen and Soderman, 1973; Soderman et al., 1973). Because of the unique buoyancy of these relatively large cells (10–100 μ in diameter) in physiological media, we were able to utilize the binding capacities of various insulin-Sepharose preparations for the development of an affinity binding buoyant density assay to study the insulin receptors on intact adipocytes (Soderman et al., 1973). It was suggested at that time that such immobilized hormone preparations may be used to fractionate heterogeneous cell populations according to their hormone sensitivities.

Several lines of evidence suggested the existence of distinct glycoprotein-like components on the adipocyte that may be responsible for the binding demonstrated by the buoyant density assay. This was indicated by the ability of a protein-containing fraction from detergent-solubilized membranes to be (a) precipitated with Con A, (b) redissolved with MeMan, (c) capable of readily binding to Con A-Sepharose affinity columns, and (d) eluted with MeMan.

The advantages of utilizing immobilized Con A for studies of Con A binding sites and surface membrane structure and behavior have been pointed out by Edelman et al. (1971) and Rutishauser et al. (1974), who used nylon fibers to fractionate thymocytes and erythrocytes. However, although they found that these cells would specifically bind to Con A fibers of Con A-agarose beads, the cells could not be released or eluted simply by the addition of competitive binding inhibitors. To circumvent this limitation, carefully controlled mechanical removal of the cells from the fibers was required (Edelman et al., 1971). It was suggested that this virtually irreversible binding may have been due to the formation of secondary strong adhesions that could be broken only when those cells were distorted (Edelman et al., 1971; Rutishauser et al., 1974).

In the present study, the binding of adipocytes to Con A-Sepharose beads could be specifically inhibited or reversed by MeMan and other saccharide inhibitors, as well as with free Con A. Analogous to our earlier study of the insulin receptor utilizing the affinity binding buoyant density procedure (Soderman et al., 1973), the binding (flotation of Con A- (or insulin-)Sepharose beads bound to low density cells), the inhibition and the reversal of binding (sedimentation of beads and high density cell-bead complexes) could readily be measured. The relative ease in reversal of binding demonstrated in the present study in contrast to the irreversibility seen in previous studies could possibly be explained by unique properties of adipocytes (e.g., buoyancy, morphology, and membrane structure) conducive to release of cells from beads, differences in conditions, and an absence or lack of interference by the above-mentioned putative secondary adhesions when using adipocytes.

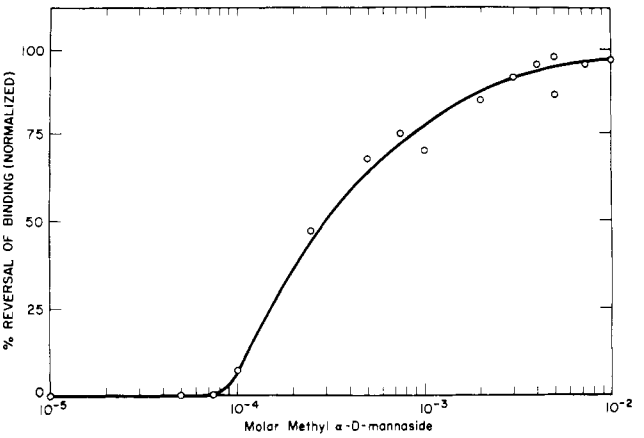

FIGURE 2: Dose-response effect of methyl α-D-mannopyranoside to reverse the binding of Con A-Sepharose to adipocytes according to affinity binding buoyant density method. Prior to the addition of saccharide to each reaction tube, beads and cells (at a ratio of 0.6 of cells to beads) were mixed and allowed to bind to each other spontaneously for 10 min. At the end of this time, greater than 97% of the beads were found to be firmly bound to, and floating with, the cells in each tube. Following this initial binding step, a 0.1-ml aliquot of saccharide, for each designated final concentration, was added to each tube, and the resultant mixtures were each rapidly remixed gently but completely and allowed to separate for 10 min into three layers (floating, intermediate, and sediment). The radioactivity associated with the beads in each sediment was then counted and compared (normalized) to the percentage that sedimented (about a 5% base line value due to the mechanical effects of the remixing step) in the absence of saccharide. Thus 0% (no reversal of binding) corresponds to the amount of radioactivity that sedimented in the absence of saccharide and 100% (complete reversal) represents sedimentation of the total radioactivity. Other details are given under Materials and Methods.

Con A had previously been shown to have pronounced effects on the mobility of its receptors on the surface of several different cell types (Unanue et al., 1972; Yahara and Edelman, 1972). Local or global movements of Con A receptors on cell surfaces, leading to clustering or aggregation of these binding sites, have been referred to as "patch" or "cap" formation. In order to gain some insight into the topographical distribution of the Con A binding sites on the adipocyte surface resulting from cell-bead interactions, microscopic examinations were made of the distribution of Con A-Sepharose beads surrounding the cells to which they were bound. Because these binding sites always appeared to be evenly distributed on the surface of the cells, and this distribution appeared no different than the distribution of sites around each bead (i.e., cells bound to immobilized Con A molecules presumably evenly distributed on the surface of the beads), it is suggested that neither Con A-Sepharose induced capping (global movements of receptors to a single region of the cell surface) nor macropatching (local movements to a few regions) occurred on the adipocyte under these conditions. Either of these events would have been expected to restrict the binding of beads to localized regions of the cell, a circumstance not apparent during frequent observations.

Previous studies (Rutishauser et al., 1974) have suggested that movements of cell receptors induced by certain immobilized ligands may be related to morphological changes in the cell. In view of this, the present finding that binding of Con A-Sepharose beads to adipocytes occurs without apparent morphological alterations in the shape of these cells

would be consistent with the suggestion that the immobilized Con A induced no gross topographical redistribution of the Con A receptors. However, the present evidence with Con A immobilized on a solid matrix does not exclude the possibility that soluble (free) Con A molecules may nevertheless be capable of modifying the distribution of receptors on these cells.

Because of the rapidly expanding interest in the use of Con A as a cell membrane probe, the affinity binding buoyant density principle has been extended here to the study of this lectin, with the adipocyte as the target cell. The action of Con A on adipocytes would appear particularly interesting in view of the previously reported insulin-like activity exhibited by Con A's interaction with these cells (Czech and Lynn, 1973; Cuatrecasas and Tell, 1973). While Czech and Lynn have attributed this effect to a nonspecific action of the lectin on the cell, others have supported a direct action on the insulin receptor (Cuatrecasas and Tell, 1973), or a general membrane effect of the lectin on the insulin receptor (De Meyts et al., 1974). The question of this site or sites of action remains unresolved. The buoyant density method may be particularly applicable to the study of this action of Con A. The feasibility of using insulin-Sepharose to examine the possible mobility of insulin receptors on adipocytes was previously suggested (Katzen, 1974).

The sensitivity of the buoyant density method for insulin receptors is indicated by the low concentration of trypsin (1 μg/ml) found to reduce the binding capacity of the adipocytes. Previous methods required 1 mg/ml to inactivate insulin receptors on these cells (Kono and Barham, 1971). The present findings that trypsin had no effect on the total binding capacity of these cells agree with the experimental results of Czech et al. (1974) who used the [^{125}I]insulin binding method. However, contrary to their conclusions, we do not believe that this can be taken to mean that insulin receptors are distinct from any of the many classes of Con A receptors. Since there are likely no more than 5×10^4 insulin receptors per cell (Gammeltoft and Gliemann, 1973), but possibly 2×10^6 total Con A receptors per cell (Edelman, 1974), tryptic destruction of insulin receptors should have only an insignificant effect on the net binding capacity for Con A.

Acknowledgment

The authors are greatly indebted to Dr. Harry Carter of this Institute for his advice on, and operation of, the Nomarski interference contrast microscope, and for the resultant photographs.

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